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Different Aluminum Tolerance among Indica, Japonica and Hybrid Rice Varieties

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Abstract: Hydroponic cultures were conducted to compare the aluminum (Al) tolerance among different rice (*Oryza sativa* L.) varieties, including indica, japonica and their hybrids. The results showed that the root growth of rice plant was inhibited in different degrees among Al treated varieties. The Al tolerance observed through relative root elongation indicated that five japonica varieties including Longjing 9, Dharial, LGC 1, Ribenyou and Koshihikari were relatively more tolerant than indica varieties. Most indica varieties in this study, such as Aus 373 and 9311 (awnless), were sensitive to Al toxicity. The Al tolerance of most progenies from japonica × indica or indica × japonica crosses was constantly consistent with indica parents. The differences of Al tolerance among Longjing 9 (japonica), Yangdao 6 (indica) and Wuyunjing 7 (japonica) were studied. Biomass and the malondial-dehyde content of Yangdao 6 under Al exposure decreased and increased, respectively, while there was no significant effect on those of Longjing 9 and Wuyunjing 7. Remarkable reduction of root activities was observed in all these three rice varieties. Significantly higher Al content in roots was found in Yangdao 6 compared to Longjing 9 or Wuyunjing 7.

Key words: aluminum tolerance; rice; japonica; indica; hybrid

Aluminum (Al^{3+}) is the most abundant metal in the earth's surface, comprising approximately 7% of the soil (Wolt, 1994). At low pH, Al is solubilized as phytotoxic Al^{3+} from non-toxic Al silicates and oxides (Hoekenga et al, 2003). Approximately 30% of the total land in the world and over 50% of potentially arable soils are acidic (Kochian et al, 2004). Al toxicity is an important factor limiting crop productivity in acidic soils (Samac and Tesfaye, 2003; Guo et al, 2013). The most significant symptom of Al toxicity is inhibition of root elongation (Huang et al, 2013), thus resulting in an adverse effect on the ability of a plant to acquire both water and nutrients (Kochian, 1995; Famoso et al, 2010). Root tolerance index, calculated as the maximum root length in the Al treatment divided by the maximum root length in the control, has been suggested to be one of the most important markers when screening genotypes and cultivars for Al toxicity (Taylor and Foy, 1985; Wu et al, 1997). Famoso et al (2010) reported a variation of Al tolerance index ranging from 0.15 to 0.97 for relative root growth among 23 rice genotypes when rice seedlings were exposed to 160 μmol/L Al.

As a critically important food crop, rice has been reported to be the most Al tolerant cereal crop under field conditions (Foy, 1988). In recent years, many studies on physiological mechanisms of Al tolerance in plants have been conducted. Organic acids with Al chelating ability play important roles in the detoxification of Al in both external and internal plants (Ma, 2000). Shen et al (2002) demonstrated that complexation with oxalate and sequestration into

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vacuoles play an important role in detoxification of Al in buckwheat leaves. It was found that Al and Al activated organic acids can be excluded from the root tip (Pellet et al, 1996; Ma et al, 2001; Kochian et al, 2004). Wang et al (2006) reported that rhizosphere pH has a positive effect on Al tolerance, and high pH can reduce Al activity and toxicity. However, the Al tolerance mechanism of rice in acid soils is still poorly understood. Studies have identified that increases in Al accumulation in the root apex of rice cause no changes of organic acid in root exudates or rhizosphere pH (Ma et al, 2002; Yang et al, 2008), which suggests that there may be other mechanism in Al tolerant rice. Cultivated rice is characterized by deep genetic divergence between the two major varietal groups, indica and japonica (Dally and Second, 1990; Garris et al, 2005; Hu et al, 2006; Londo et al, 2006). It was found that Al tolerance in japonica is higher than that in indica (Famoso et al, 2011). After exposure to 50 μmol/L Al for 24 h, root elongation is inhibited by 42% for Koshihikari (japonica variety), while 73% for Kasalath (indica variety) (Ma et al, 2002). Previous studies make it attractive to transfer Al tolerance genes in japonica to the sensitive rice varieties, whereas Al tolerance of hybrids has not been studied widely.

The objectives of the present study were to compare the Al tolerance of 43 rice genotypes, including indica, japonica and their hybrids, and to study the differences in Al tolerance among Longjing 9 (japonica), Yangdao 6 (indica) and Wuyunjing 7 (japonica).

MATERIALS AND METHODS

Rice materials

Forty three rice genotypes provided by Rice Research Institute, Jiangxi Academy of Agricultural Sciences, China (Table 1), and two popular varieties, Wuyunjing 7 (japonica) and Yangdao 6 (indica), provided by Soil Research Institute, Chinese Academy of Science, were used in the present study.

Hydroponics and Al treatment

Seeds of rice were surface sterilized in 0.1% NaClO for 15 min, rinsed and soaked in water at 30 ºC in darkness for 24 h. The soaked seeds were then transferred to a plastic mesh floated on 0.5 mmol/L $CaCl_2$ solution. The seeds were cultured in darkness for 48 h and then in a controlled growth chamber for 48 h, with 14 h light [200 μmol/(m^2·s)] at 30 ºC and 10 h dark at 25 ºC, respectively, and the solution was renewed every day. On 5 and 10 d, uniform seedlings were grown in 50 μmol/L $AlCl_3$ solution (containing 0.5 mmol/L $CaCl_2$) for 24 h. The treatment of 0.5 mmol/L $CaCl_2$ without Al served as the control. The root lengths of rice seedlings were measured before and after Al treatment.

Four-day-old seedlings were cultured in 50% strength Kimura B solution for 6 d. Then the seedlings were interval cultured with Al for 12 d, 100% Kimura B solution for 1 d then cultured with Al (200 μmol/L $AlCl_3$ and 0.5 mmol/L $CaCl_2$) and without Al (0.5 mmol/L

Table 1. Genetic background of different rice germplasms.

Label	Designation	Generation	Subspecies	Label	Designation	Generation	Subspecies
09001	Dakanala	Parent	japonica	09030	IR70369B	Parent	indica
09003	Dharial	Parent	japonica	09031	IR73013-95-1-3-2R	Parent	indica
09004	LGC1	Parent	japonica	09032	IR73885-1-4-3-2-1-10R	Parent	indica
09006	Longjing 9	Parent	japonica	09033	IR78371B	Parent	indica
09009	Ribenyou	Parent	japonica	09034	IR79156B	Parent	indica
09010	Koshihikari	Parent	japonica	09035	IR29723-143-3-2-1R	Parent	indica
09012	Srt 1	Parent	japonica	09136	LGC1/Dali	Advanced generation	japonica × japonica
09014	Dali	Parent	japonica	09146	LGC1/Dali	Advanced generation	japonica × japonica
09016	Aus 373	Parent	indica	09158	Koshihikari/LGC1	Advanced generation	japonica × japonica
09017	Aus 373	Parent	indica	09166	Srt 1/Koshihikari	Advanced generation	japonica × japonica
09018	9194	Parent	indica	09177	Srt 1/Ribenyou	Advanced generation	japonica × japonica
09019	Ganwanxian 32	Parent	indica	09194	9194/LGC1	Advanced generation	indica × japonica
09020	Ganwanxian 9	Parent	indica	09204	Srt 1/9194	Advanced generation	japonica × indica
09021	Ganwanxian 30	Parent	indica	09233	Srt 1/9194	Advanced generation	japonica × indica
09022	9311 (awned)	Parent	indica	09280	Koshihikari/9194	Advanced generation	japonica × indica
09023	9311 (smooth)	Parent	indica	09297	Ganwanxian 32/Ribenyou	Advanced generation	indica × japonica
09024	Ganzaoxian 58	Parent	indica	09317	Srt 1/Dali	Advanced generation	japonica × japonica
09025	Ganzaoxian 59	Parent	indica	09364	9194/Ribenyou	Advanced generation	indica × japonica
09026	Lijiangheigu	Parent	indica	09365	Dongye/Koshihikari	Advanced generation	japonica × japonica
09027	Doongara	Parent	indica	Fan 12	Koshihikari/9194	Strain	japonica × indica
09028	IR58025B	Parent	indica	Fan 13	Koshihikari/9194	Strain	japonica × indica
09029	IR60819-34-2R	Parent	indica				

$CaCl_2$) for 1 d; this procedure was repeated six times. The nutrient solution (pH 4.5) contained 0.18 mmol/L $(NH_4)_2SO_4$, 0.27 mmol/L $MgSO_4·7H_2O$, 0.09 mmol/L KNO_3, 0.18 mmol/L $CaNO_3·4H_2O$, 0.09 mmol/L KH_2PO_4, 20 µmol/L Na_2EDTA-Fe(II), 9 µmol/L $MnCl_2·4H_2O$, 46 µmol/L H_3BO_3, 9 µmol/L $Na_2MoO_4·4H_2O$, 0.7 µmol/L $ZnSO_4·7H_2O$, and 0.3 µmol/L $CuSO_4·5H_2O$.

Measurements and chemical analysis

Relative root elongation (RRE)

RRE was used for estimating Al tolerance in 43 rice genotypes. The formula is shown as below:

$RRE\ (\%) = (RL_{\mathrm{Al+}} - RL_{\mathrm{Al\text{-}0}}) / (RL_{\mathrm{CK+}} - RL_{\mathrm{CK\text{-}0}}) \times 100$

$RL_{\mathrm{Al+}}$ and $RL_{\mathrm{Al\text{-}0}}$ are the lengths of the longest root after and before Al treatment, respectively, and $RL_{\mathrm{CK+}}$ and $RL_{\mathrm{CK\text{-}0}}$ are the lengths of the longest root of the control after and before treatment, respectively (Watanabe and Okada, 2005).

Dry biomass

Sampled seedlings were washed carefully, and then dried at 105 ºC in oven for 30 min. Dry biomass was measured when dried seedlings reached a constant weight at 80 ºC.

Root activities

Root activity directly reflects the growth conditions of plant, and therefore, it is an essential index. It was measured by triphenyl tetrazolium chloride (TTC) deoxidization intensity (Lin et al, 2001). Roots (0.2 g) were dipped in a mixture of 5 mL 0.4% TTC and 5 mL phosphate buffer for 1 to 3 h at 37 ºC, and then 2 mL of 1 mol/L H_2SO_4 was added to terminate the reaction. The roots were sampled and ground with 3–4 mL methyl ethanoate, and diluted to 10 mL. The absorbance at 485 nm of the supernatant was determined using spectrophotometer (UV-2450, SHIMADZU, Tokyo, Japan).

Estimation of lipid peroxidation

Lipid peroxidation was determined according to Duan et al (2005). Fresh roots and leaves (about 0.5 g) were ground in 5 mL of 5% trichloroacetic acid. The homogenate was centrifuged at 3 000 r/min for 10 min. The mixture of 2 mL supernatant and 2 mL of 0.67% 2-thiobarbituric acid was boiled for 30 min, and then centrifuged after cooling down. The malondialdehyde (MDA) content (C, µmol/g) was calculated by the absorbance at 450, 532 and 600 nm according to the formula: $C = [6.45 \times (A_{532} - A_{600}) - 0.56 \times A_{450}] \times 5 / 0.5$.

Mineral element concentrations

The dried plant samples (0.2 g) were ground and digested with $HNO_3 : HClO_4$ (87 : 13), and inductively coupled plasma-atomic emission spectrometer (ICP-AES) was used to determine the concentrations of the elements (Chen et al, 2006).

Statistical analysis

One-way analysis of variance was used to confirm the variability of data and validity of results, and the difference between treatments was determined using Duncan's test at $P < 0.05$ level. Dendrogram was analyzed by average linkage using rescaled distance.

RESULTS

Effects of Al stress on root elongation

In general, Al inhibits root growth. RRE results revealed that after exposure to 50 µmol/L Al for 24 h, root growth of 09017 was significantly inhibited, its RRE being 7.35% of the control, and the highest RRE

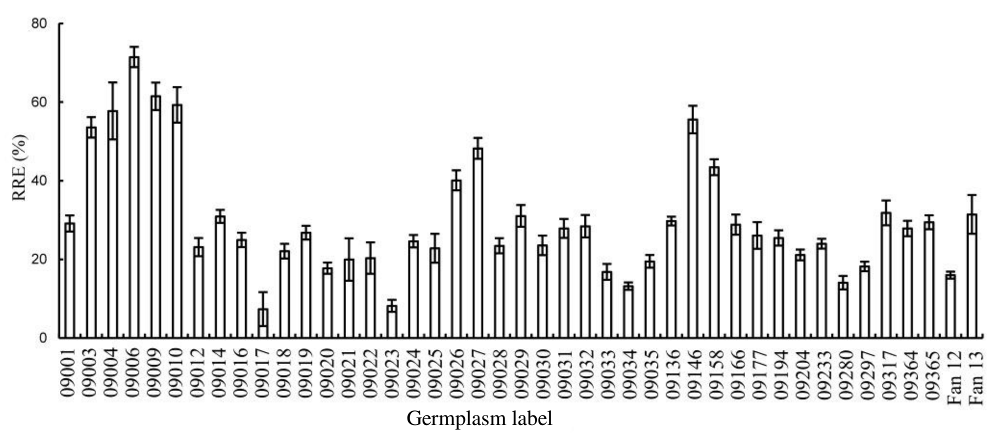

Fig. 1. RRE (relative root elongation) of different rice genotypes under aluminum (50 µmol/L) stress for 24 h (means ± *SE*, *n* = 10).

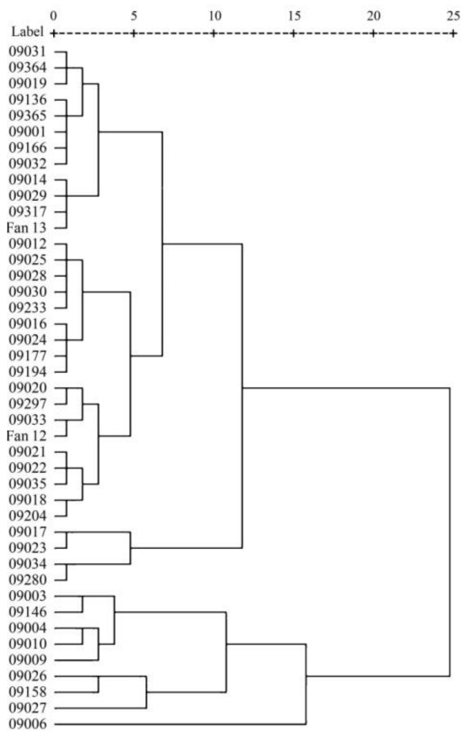

Fig. 2. Clustering figure of RRE (relative root elongation) of different rice genotypes based on rescaled distance.

(71.47%) was found in 09006 (Fig. 1). According to the analysis of RRE clustering, five japonica varieties 09003, 09004, 09006, 09009 and 09010 were tolerant. Al tolerance levels of 09026, 09027, 09146 and 09158 were medium, and the others were sensitive to Al (Fig. 2). The results indicated that Al tolerance level of 09006 was the highest among the 43 rice genotypes, and as a whole, japonica genotypes were more tolerant to Al than indica ones.

Significant difference in RRE of parents and offsprings

As shown in Figs. 3 and 4, RRE of japonica and indica hybrids had no significant difference with indica but it was evidently different from japonica. Hybrids 09204 and 09233 were exceptions, which were not distinct from either japonica or indica (Fig. 3). Al tolerance of the offsprings approached the sensitive parent. When the parents were both japonica, the Al tolerance of the hybrids showed no genetic regulation (Fig. 5). For example, Al tolerance of 09146, 09166 and 09177 was consistent with the female parents, whereas 09136 and 09317 had similar Al tolerance to their male parents, and 09158 showed a divergence with the parents.

Growth and physiological effects of Al stress

Effects of Al stress on biomass accumulation

According to the previous results, 09006 (Longjing 9) was chosen to study physiological effects of Al on rice seedlings, and also to compare with two popular varieties, Wuyunjing 7 and Yangdao 6, which are cultivated widely in south and north of Jiangsu Province, China, respectively. As shown in Fig. 6, after being exposed to 200 μmol/L Al for 12 d, biomasses of Wuyunjing 7 and Longjing 9 showed no significant decrease compared with the treatment without Al. The biomass of shoots and roots in Yangdao 6 decreased by 29.7% and 15.0% compared with the treatment without Al, respectively.

Effects of Al stress on roots

As usual, root activity was measured by TTC

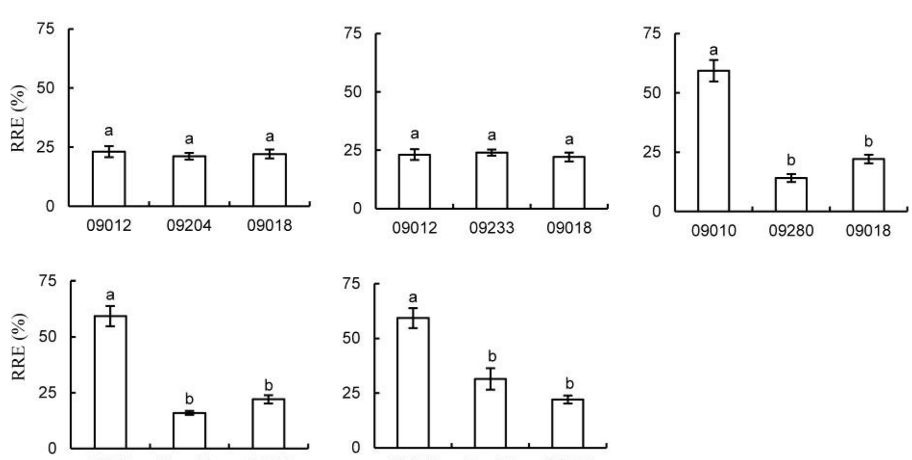

Fig. 3. RRE (relative root elongation) of japonica × indica hybrids and their parents under aluminum (50 μmol/L) stress for 24 h (means ± *SE*, *n* = 10).
Different letters mean significant difference at $P < 0.05$, according to the Duncan's test.

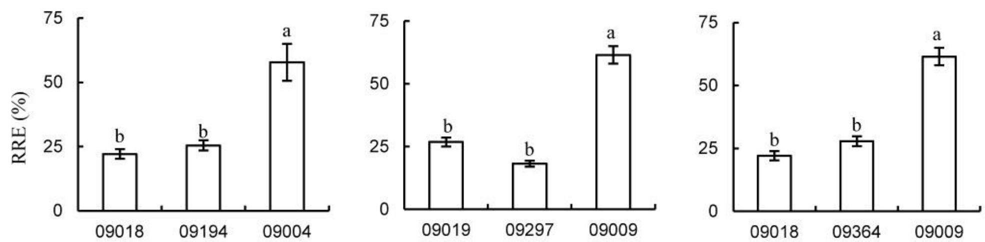

Fig. 4. RRE (relative root elongation) of indica × japonica hybrids and their parents under aluminum (50 μmol/L) stress for 24 h (means ± *SE*, *n* = 10).

Different letters mean significant difference at $P < 0.05$, according to the Duncan's test.

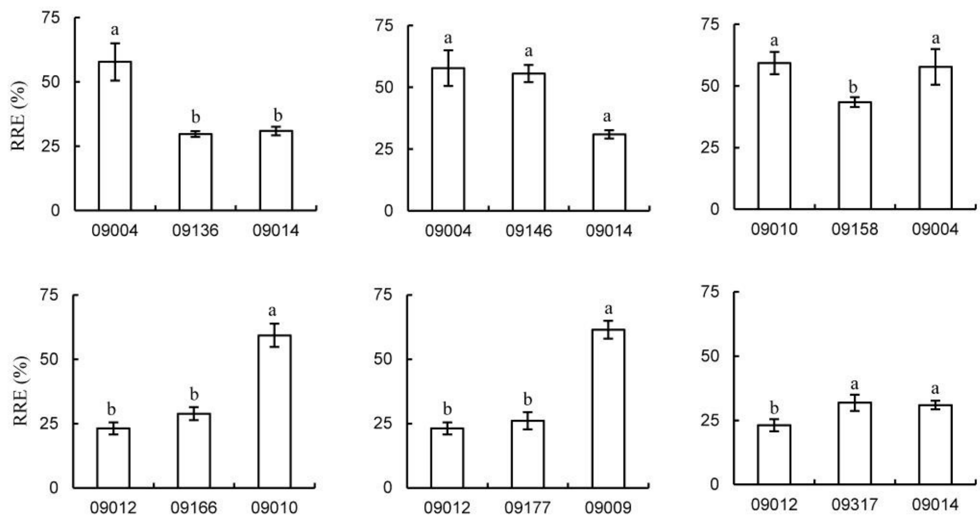

Fig. 5. RRE (relative root elongation) of japonica × japonica hybrids and their parents under aluminum (50 μmol/L) stress for 24 h (means ± *SE*, *n* = 10).

Different letters mean significant difference at $P < 0.05$, according to the Duncan's test.

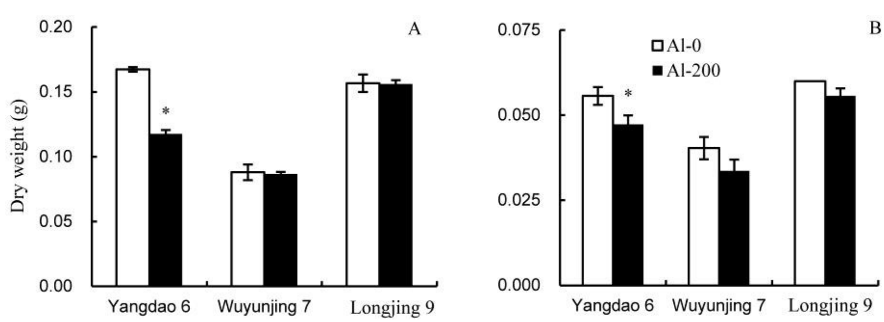

Fig. 6. Biomass of shoots (A) and roots (B) for three rice varieties after exposure to 200 μmol/L aluminum (Al) for 12 d (means ± *SE*, *n* = 3).

* means significant difference at $P < 0.05$, according to the Duncan's test.

deoxidization intensity. In the plant control (without Al treatment), root activities of the three varieties showed no significant differences with each other. When exposed to Al for 12 d, root vigor was remarkably affected. The root activities of Yangdao 6, Wuyunjing 7 and Longjing 9 decreased by 83.7%, 35.0% and 45.3%, respectively (Fig. 7).

The root activities in Wuyunjing 7 and Longjing 9 were approximately 4.97 and 3.53 times higher than that in Yangdao 6 under Al exposure.

Effects of Al stress on lipid peroxidation

Lipid peroxidation was observed through MDA content. There was no significant difference found in the three rice varieties. Over-exposed to Al for 12 d, The MDA contents in leaves of Yangdao 6 increased significantly by 52.6% ($P < 0.05$) (Fig. 8). The MDA contents in shoots of Wuyunjing 7 and Longjing 9 were not obviously different from the Al-0 treatment ($P > 0.05$).

MDA contents in roots of Wuyunjing 7 and

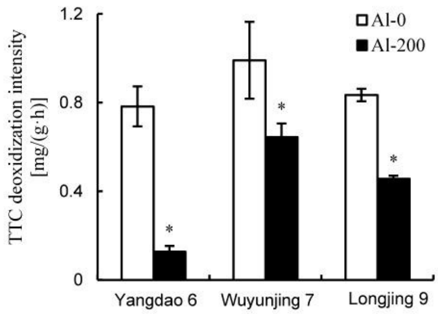

Fig. 7. Triphenyl tetrazolium chloride (TTC) deoxidization intensity of three rice varieties after exposure to 200 μmol/L aluminum (Al) for 12 d (means ± *SE*, *n* = 3).

* means significant difference at $P < 0.05$, according to the Duncan's test.

Longjing 9 were not significantly changed compared with the control (Fig. 8-B). The MDA levels in roots of Yangdao 6 showed a marked increase of 38.5%.

Al content in different rice varieties

In the Al-0 treatment, Al contents in shoots of three rice varieties were similar (Fig. 9-A). After exposure to Al for 12 d, the Al levels in shoots of the three varieties showed no significant differences from that of the control.

The Al levels in roots of the three varieties increased significantly after 12 d exposure to Al (Fig. 9-B). Al content in roots of Yangdao 6 was markedly higher than those of Wuyunjing 7 and Longjing 9. There was no significant difference in Al accumulation in roots of Wuyunjing 7 and Longjing 9. Al content in roots of Yangdao 6, Wuyunjing 7 and Longjing 9 were 54.6, 21.8 and 26.1 times higher than those in shoots, respectively. These results showed that the root is the main tissue where Al accumulates, which has been previously shown by Silva et al (2013).

Effects of Al stress on mineral element contents

As shown in Table 2, phosphorus (P) level in roots of Yangdao 6 decreased significantly, while P levels in roots of Wuyunjing 7 and Longjing 9 increased markedly ($P < 0.05$). After 12 d exposure to Al, potassium (K) and calcium (Ca) contents in roots of the three varieties decreased significantly ($P < 0.05$); magnesium (Mg) levels in roots of Yangdao 6 and Wuyunjing 7 were also affected significantly ($P < 0.05$); whereas manganese (Mn) content in roots of Yangdao 6 declined markedly ($P < 0.05$).

P content in shoots of the three varieties was not affected by Al exposure (Table 3). When exposed to Al for 12 d, K content in shoots of Longjing 9 reduced significantly ($P < 0.05$); contents of Ca and Mg decreased evidently in shoots of Yangdao 6 and Wuyunjing 7 ($P < 0.05$), whereas Mn levels in shoots of the three varieties all showed marked differences compared to Al-0 treatment.

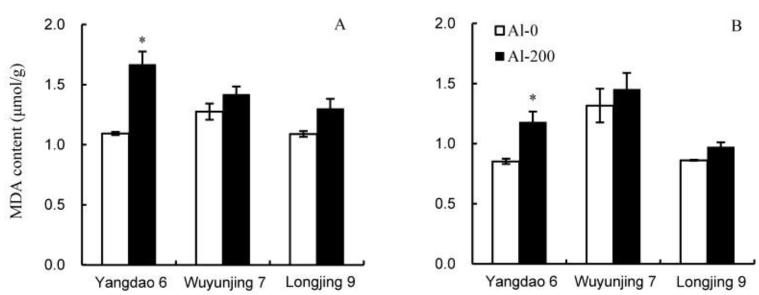

Fig. 8. Malondialdehyde (MDA) content in shoots (A) and roots (B) of three rice varieties after exposure to 200 μmol/L aluminum (Al) for 12 d (means ± *SE*, *n* = 3).

* means significant difference at $P < 0.05$, according to the Duncan's test.

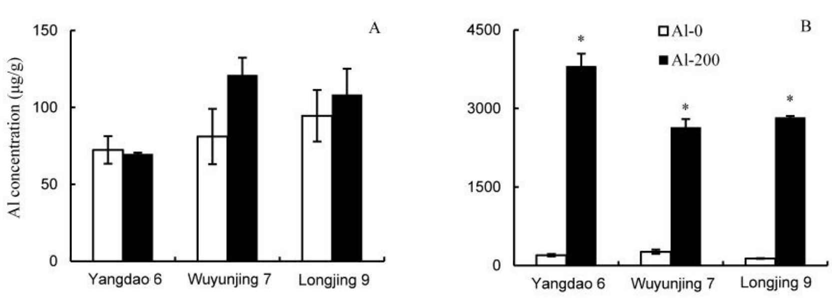

Fig. 9. Aluminum (Al) concentration in shoots (A) and roots (B) of three rice varieties after exposure to 200 μmol/L Al for 12 d (mean ± *SE*, *n* = 3).

* means significant difference at $P < 0.05$, according to the Duncan's test.

Table 2. Effects of aluminum (Al) (200 μmol/L) stress on phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg) and manganese (Mn) contents in roots of three rice varieties. (mg/g)

Element	Yangdao 6		Wuyunjing 7		Longjing 9	
	Al-0	Al+	Al-0	Al+	Al-0	Al+
P	5.83 ± 0.17	5.10 ± 0.24	6.17 ± 0.05	7.08 ± 0.50 *	4.75 ± 0.11	6.46 ± 0.21 *
K	11.39 ± 0.18	7.55 ± 0.65 *	9.40 ± 0.20	6.36 ± 0.35 *	12.93 ± 0.32	10.59 ± 0.02 *
Ca	1.90 ± 0.12	1.16 ± 0.08 *	1.95 ± 0.09	1.47 ± 0.16 *	2.39 ± 0.03	1.19 ± 0.05 *
Mg	2.98 ± 0.27	1.39 ± 0.19 *	1.82 ± 0.07	0.97 ± 0.07 *	1.32 ± 0.01	0.88 ± 0.04
Mn	0.06 ± 0.01	0.04 ± 0.00 *	0.06 ± 0.00	0.05 ± 0.01	0.07 ± 0.00	0.06 ± 0.00

The date are means ± *SE* (n = 3).

* means significant differences after Al treatment, according to the Duncan's test ($P < 0.05$).

Table 3. Effects of aluminum (Al) (200 μmol/L) stress on phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg) and manganese (Mn) contents in shoots of three rice varieties. (mg/g)

Element	Yangdao 6		Wuyunjing 7		Longjing 9	
	Al-0	Al+	Al-0	Al+	Al-0	Al+
P	8.42 ± 0.52	7.54 ± 0.28	11.11 ± 0.35	11.07 ± 0.82	8.50 ± 0.23	7.30 ± 0.14
K	20.03 ± 1.09	19.83 ± 0.62	20.41 ± 0.40	19. 56 ± 1.20	18.75 ± 0.07	15.36 ± 0.22 *
Ca	5.31 ± 0.60	3.28 ± 0.10 *	4.60 ± 0.23	3.77 ± 0.13	3.39 ± 0.05	2.85 ± 0.02
Mg	6.77 ± 0.41	4.20 ± 0.14 *	5.08 ± 0.19	4.19 ± 0.13 *	3.19 ± 0.03	3.31 ± 0.08
Mn	0.44 ± 0.01	0.27 ± 0.02 *	0.67 ± 0.02	0.53 ± 0.02 *	0.31 ± 0.00	0.24 ± 0.00 *

The date are means ± *SE* (n = 3).

* means significant differences after Al treatment, according to the Duncan's test ($P < 0.05$).

DISCUSSION

Although rice is generally considered to be the most tolerant to Al stress among the cereal crops, its genotypic variations in Al tolerance have been reported (Khatiwada et al, 1996; Ma et al, 2002; Famoso et al, 2010, 2011). Kikui et al (2005) reported that rice possesses an Al-tolerant function which is under genetic control and specifically operates for root growth at the germination stage. So it is very important to understand the differences in Al tolerance among rice genotypes. One of the best ways to decrease Al toxicity in acid soils is to develop and apply rice varieties with high Al tolerance. Since the crosses between the tolerant parents had higher Al tolerance than those involving Al-susceptible parents (Wu et al, 1997).

In order to further understand the mechanisms of Al toxicity in rice, 43 rice genotypes were studied. In the present study, five high Al-tolerant rice varieties were identified based on RRE under Al exposure (Figs. 1 and 2). The results indicated that japonica variety showed higher Al tolerance compared to the indica one, which is consistent with the results of Ma et al (2002) and Famoso et al (2010). It was reported that Koshihikari (japonica) shows higher Al tolerance compared to Kasalath (indica) (Ma et al, 2002). Famoso et al (2010) represented the genetic and Al tolerance diversity of the indica and japonica varietal groups for Al tolerance experiments using 23 rice genotypes, in which they found the indica varietal group (n = 12) is generally more susceptible than the japonica one (n = 11), with mean tolerance values of 0.42 and 0.69, respectively. Famoso et al (2010) demonstrated that the high levels of Al tolerance in rice are mediated by a novel mechanism that is independent of root tip Al exclusion. So far, the mechanism of Al tolerance in rice is unclear.

The hybrid varieties were also studied (Figs. 3–5). We found that whether the hybrid derived from japonica × indica or indica × japonica, Al tolerance of the hybrid was constantly consistent with the indica variety, which means Al tolerance in the hybrid varieties biased towards the sensitive parent. This is consistent with a report by Wu et al (1997) which indicates that the tolerance degree of F_1 hybrids was influenced by the susceptible parent genotype. So, it is difficult to obtain new rice varieties with high Al tolerance by the conventional japonica × indica hybrid approach. This investigation has been documented for the first time in this study.

Previous results revealed that the *ASR5* expression levels were not affected by Al treatment in the Al-sensitive indica variety Taim, but were significantly increased in the Al-tolerant japonica rice variety Nipponbare (Arenhart et al, 2013). Although the results suggested that ASR5 protein acts as a transcription factor to regulate the expression of different genes that collectively protect rice cells from Al induced stress responses (Arenhart et al, 2013), the detailed and complex Al tolerance mechanisms among indica and japonica are largely unknown.

In order to reveal the Al tolerance mechanism in rice varieties, it is necessary to make crosses applying two greatly different Al tolerant rice genotypes as parental species, construction of separate groups, and then by map-based cloning, to identify more Al tolerance genes to further reveal the Al tolerance mechanism in rice. The method used for screening is also very important. Narasimhamoorthy et al (2007) suggested that a combination of soil-based screening and hydroponics may be essential. If there are several Al tolerance mechanisms in rice plants, they should be controlled by different genes, and a single screening method fails to demonstrate all of the information.

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